Additions to *Neopestalotiopsis* (Amphisphaeriales, Sporocadaceae) fungi: two new species and one new host record from China

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Abstract

Background

In this study, three *Neopestalotiopsis* taxa were identified, associated with leaves of *Zingiber officinale*, *Elaeagnus pungens* and *Salacca zalacca*.

New information

Based on morphology and multi–gene analyses of the internal transcribed spacer (ITS), beta-tubulin (TUB2) and translation elongation factor 1–alpha (TEF1), the five strains of *Neopestalotiopsis* represent two novel and one known species. They are introduced with descriptions, illustrations and notes herein.

Keywords

morphology, Pestalotiod, phylogeny, taxonomy, Zingiberaceae

Introduction

Pestalotiod fungi distribute commonly as saprobes, pathogens and endophytes, which can cause a variety of plant diseases (Huanaluek et al. 2021). Most of this fungal group lack sexual morphs and only 13 species can reproduce the sexual stage (Maharachchikumbura et al. 2011, Nozawa et al. 2017). Pestalotioid fungi are placed in

Sporocadaceae (Amphisphaeriales) (Jayawardena et al. 2019, Wijayawardene et al. 2020). Based on the conidia pigment colour, conidiophores and molecular phylogeny, *Neopestalotiopsis* was segregated from the old *Pestalotiopsis* genus (Maharachchikumbura et al. 2014a). *Neopestalotiopsis, Pestalotiopsis* and *Pseudopestalotiopsis* differ from each other by the colour of the conidial three median cells (Maharachchikumbura et al. 2014a). However, the delimitation of species, only through phenotypic characteristics, is difficult (Maharachchikumbura et al. 2016), thus morphological and phylogenetic approaches should be combined to determine the new taxa. Seventy-two species of *Neopestalotiopsis* are recorded in Index Fungorum (2022), but only forty-one species of *Neopestalotiopsis* are accepted, based on molecular data (Jayawardena et al. 2019). In this paper, two new species and a new Chinese record of *Neopestalotiopsis* found on *Zingiber officinale* Rosc., *Elaeagnus pungens* Thunb. and *Salacca zalacca* (Gaertn.) Voss. in Zingiberaceae are described and illustrated.

Materials and methods

Sample collection and fungal strains isolation

Diseased fresh leaf samples were collected from *Z. officinale*, *E. pungens* and *S. zalacca* in Hainan Province, China in 2020. Fresh specimens were taken to the laboratory in paper envelopes. The strains were obtained using single spore isolation, following Senanayake (2020). Once the single spore germinated, it was transferred to potato dextrose agar (PDA) and cultured at room temperature (24°C).

Morphological description

Microscopic slides were prepared with lactic acid and examined using an Axioscope 5 with Axiocam 208 colour (ZEISS, Oberkohen, Germany) at 1000× magnification. The morphology of fungi was photographed by the camera. Photo–plates were made by Adobe Photoshop CS6, with the Tarosoft (R) Image Frame Work programme being used for measurements. Herbaria materials were deposited in the Herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University (HGUP). Cultures were deposited to the Culture Collection of the Department of Plant Pathology, Agriculture College, Guizhou University (GUCC) (Table 1). The taxonomic information of new species was submitted to MycoBank (http://www.mycobank.org).

DNA extraction, PCR reaction and sequencing

The fresh mycelia were scraped off with a sterilised scalpel when colonies reached 80 mm in diameter. Genomic DNA was extracted using the Fungus Genomic DNA Extraction Kit (Biomiga GD2416), following the manufacturer's instructions. Polymerase chain reactions (PCR) were performed in a 20 μl reaction volume: 1 μl of DNA template, 1 μl of each forward and reverse primers, 10 μl of 2× Bench Top^{TM}Taq Master Mix and 7 μl double–distilled water (ddH_2O). The partial internal transcribed spacer (ITS) rDNA was amplified with the primer pair ITS4/ITS5 (White et al. 1990), TEF1 was amplified with primers EF1–728f/EF2 (O'Donnell et al. 1998, Carbone and Kohn 1999) and the TUB2 was amplified with primers T1/Bt2b (Glass and Donaldson 1995, O'Donnell and Cigelnik 1997). PCR products were sequenced by using appropriate primers for amplification reactions by SinoGenoMax, Beijing. The obtained DNA sequences were submitted to GenBank to obtain their accession numbers (Table 1). DNA base differences on three loci between our strains and ex-type or representative strains of relative *Neopestalotiopsis* taxa are shown in Table 2.

Phylogenetic analyses

The phylogeny was constructed by analyses from sequences of ITS, TEF1 and TUB2 sequence data. The fungal sequences were aligned by using the online version of MAFFT v. 7.307 (Katoh and Standley 2016) and edited by using the BioEdit programme (Hall 1999), using the SequenceMatrix 1.7.8 (Vaidya et al. 2011) to multi-source data merging. Ambiguous regions were excluded from analyses using AliView (Larsson 2014), gaps were treated as missing data and optimised manually with *Pestalotiopsis diversiseta* Maharachch. & K.D. Hyde (MFLUCC 12–0287) as the outgroup (Table 2). Combined analyses of ITS, TUB2 and TEF1 sequence data were performed. Phylogenetic analyses were constructed by Maximum Likelihood (ML), Maximum Parsimony (MP) and Bayesian Posterior Probability (BYPP) methods; they were carried out as detailed in Dissanayake et al. (2020).

Maximum Parsimony analysis was performed with PAUP v. 4.0b10 (Swofford 2002), 1000 bootstrap replicates, using heuristic search on random stepwise addition and tree bisection reconnection (TBR). Maxtrees was set to 5000. For each tree generated, consistency index (CI), retention index (RI), tree length (TL), rescaled consistency index (RC) and homoplasy index (HI) were calculated.

The Maximum Likelihood analysis was performed using the CIPRES Science Gateway web server RAxML–HPC BlackBox (Stamatakis 2014) and 1000 rapid bootstrap replicates were run with the GTR+GAMMA model of nucleotide evolution.

Bayesian Posterior Probability analyses were performed by MrModeltest v.2.3 (Nylander et al. 2004) and MrBayes 3.2 (Ronquist et al. 2012) with the Markov Chain Monte Carlo (MCMC) method. The GTR model was selected as the best model for the TUB2. The MCMC runs were launched with four chains starting from random tree topology between 1,000,000–5,000,000 generations and sampling every 100 generations. The first 5000 samples were excluded as burn–in.

Genealogical Concordance Phylogenetic Species Recognition (GCPSR) analysis

The phi-test incorporated in the SplitsTree software (Huson 1998, Huson and Bryant 2006) was used to test signals of recombination as described by Quaedvlieg et al. (2014)

. The evolutionary independence was revealed, using the GCPSR concept for the *Neopestalotiopsis* dataset with relevant taxa. In the pairwise homoplasy index (PHI), if a value below 0.05 was obtained, it provided evidence for the presence of significant recombination within a dataset. The test is proven to be a robust calculation and no previous knowledge about population history, recombination rate, mutation rate and rate heterogeneity across sites is necessary (Bruen et al. 2006).

Taxon treatments

Neopestalotiopsis elaeagni Y.K. He & Yong Wang bis, sp. nov.

- MycoBank 844750

Material

Holotype:

a. scientificName: *Neopestalotiopsis elaeagni*; order: Amphisphaeriales; family: Sporocadaceae; genus: *Neopestalotiopsis*; country: China; stateProvince: Hainan; locality: Haikou City, Leiqiong Haikou Volcano Cluster World Geopark; verbatimCoordinates: 109°39’ E, 20°13’ N; recordedBy: Yu-ke He; associatedOccurrences: GUCC 21002; identifiedBy: Yu-ke He; dateIdentified: 2020; collectionID: HGUP 10002; occurrenceID: 4AEF2BC4-2D13-5384-B974-1611E67B3932

Description

Associated with the leaf blight of *Elaeagnus pungens* Thunb. Disease symptom: A large irregular scab on the leaves of *E. pungens*, light brown, edges dark brown to reddish-brown. The boundary of the scab was not obvious. There were many black, small and punctuate conidia on the scab. Sexual morph: not observed. Asexual morph (Fig. 2): Conidiomata dark, punctiform, scattered on the host scab, 110–300 µm (n = 40), releasing black conidia. Conidiophores discrete to lageniform, hyaline, smooth– and thin–walled, 8–13 × 2–3 µm. Conidia 19–25 × 4.5–7 µm, fusiform to clavate, straight to slightly curved, 4–septate; basal cell obconic with a truncate base, hyaline or pale brown, smooth– and thin–walled, 3.5–5 µm long; three median cells 12–15 µm long, versicoloured, dark brown to light brown, septa and periclinal walls darker than the rest of the cell; second cell brown, 3.5–5.5 µm long; third cell brown, 3–5.5 µm long; fourth cell light brown 3.5–5 µm long; apical cell 3–5.5 µm long, hyaline, conic to acute, with 1–3 tubular appendages inserted at different loci, but in the same crest at the apex of the apical cell, unbranched, flexuous, 13–30 µm long; most conidia have tubular appendages or single appendage in the basal cell, hyaline, unbranched, centric, 5–7.5 µm long.

Culture characteristics: Colonies on PDA medium reaching 5–5.5 cm diam. After 10 d at 24°C, the mycelium white, cottony, odourless, soft, without exudate and round with regular edges. Under the surface of hyphal layer, releasing conidia in a black, slimy mass. The reverse side of the culture dish is smooth and light yellow.

Etymology

elaeagni, in reference to the host genus (*Elaeagnus*) from which it was isolated.

Notes

Phylogenetically, the new species is sister to *Neopestalotiopsis chrysea* (MFLUCC 12–0261), *Neopestalotiopsis umbrinospora* (MFLUCC 12–0285) and *Neopestalotiopsis asiatica* (MFLUCC 12–0286). However, *N. elaeagni* differed from *N. chrysea* by having shorter apical appendage (*N. elaeagni*: 13–30 μm vs. *N. chrysea*: 22–30 μm), differed from *N. umbrinospora* by having smaller conidia and shorter apical appendage (Conidia: *N. elaeagni*: 19–25 × 4.5–7 μm vs. *N.umbrinospora*: 19–29 × 6–8 μm; apical appendage length: *N. elaeagni*: 13–30 μm vs. *N.umbrinospora*: 22–35 μm) and differed from *N. asiatica* by having shorter apical appendage (*N. elaeagni*: 13–30 μm vs. *N. asiatica*: 20–30 μm) (Maharachchikumbura et al. 2012) (Table 3). According to the PHI analysis, our dataset showed a 1.0 value indicating no significant genetic recombination between our newly-introduced *Neopestalotiopsis* strains with other related taxa. Combined with morphology, phylogenetic analysis and PHI test results and we propose *N. elaeagni* as a novel species.

Neopestalotiopsis zingiberis Y.K. He & Yong Wang bis, sp. nov.

- MycoBank 844751

Material

Holotype:

a. scientificName: *Neopestalotiopsis zingiberis*; order: Amphisphaeriales; family: Sporocadaceae; genus: *Neopestalotiopsis*; country: China; stateProvince: Hainan; locality: Haikou City, Wuzhishan Nature Reserve; verbatimCoordinates: 109°32’ E, 18°48’ N; recordedBy: Yu-ke He; associatedOccurrences: GUCC 21001; identifiedBy: Yu-ke He; dateIdentified: 2020; collectionID: HGUP 10001; occurrenceID: 8671AAA3-BD98-5D71-9E18-6FFEF372275A

Description

Associated with leaf blight of *Zingiber officinale* Rosc. Disease symptom: A long oval to irregular, ring-like scab, light brown, edge reddish-brown, slightly sunken on adaxial surface. The boundary of the scab is obvious, with a narrow yellow halo around the scab. There are many black, small and punctuate conidia on the scab. Sexual state: unknown. Asexual morph (Fig. 3): Conidiomata is dark, oblate, scattered on the host scab, 104–202 μm. Conidiophores discrete to lageniform, hyaline, smooth– and thin–walled, annellidicae, 12–25 × 3–6 μm (n = 40). Conidia 21–31 × 6–9.5 μm, fusiform to clavate, straight to slightly, 4–septate; basal cell obconic with a truncate base, hyaline or pale brown, smooth– and thin–walled, 3–6 μm long; three median cells 15–19 µm long, septa and periclinal walls darker than rest of the cell, versicoloured, wall rugose; second cell brown, 4–6 µm long; third cell brown, 4–7 µm long; fourth cell light brown 4–6 µm long; apical cell 3–5 µm long, hyaline, conic to acute, with 1–3 tubular appendages insert at different loci, but in the same crest at the apex of the apical cell, unbranched, flexuous, 12–15 µm long; most spores have no tubular appendages or single appendage, unbranched, centric, 0–6 µm long.

Culture characteristics: Colonies on PDA medium reaching 8–9 cm diam. after 15 d at 24°C, the mycelium is yellowish or white, soft and round with irregular edges. Under the surface of hyphal layer, releasing conidia in a black, slimy mass. Dark brown pigment is deposited on the bottom of the Petri dish.

Etymology

zingiberis, in reference to the host genus (*Zingiber*) from which it was isolated.

Notes

Neopestalotiopsis zingiberis (GUCC 21001) formed a distinct clade and sistered to *Neopestalotiopsis magna* (MFLUCC 12–0652) (Fig. 1). Morphologically, conidia of *N. zingiberis* (21–31 × 6–9.5 µm) are smaller than *N. magna* (42–46 × 9.5–12 µm) and also differed by having branched, flexuous apical tubular appendages (Maharachchikumbura et al. 2014a) (Table 3). Thus, we propose *N. zingiberis* as a novel taxon.

Neopestalotiopsis samarangensis (Maharachch. & K.D. Hyde)

- MycoBank 809778

Nomenclature

Neopestalotiopsis samarangensis (Maharachch. & K.D. Hyde) Maharachch., K.D. Hyde & Crous in Maharachchikumbura, Hyde, Groenewald, Xu & Crous, Stud. Mycol. 79: 147 (2014)

Material

a. scientificName: *Neopestalotiopsis samarangensis*; order: Amphisphaeriales; family: Sporocadaceae; genus: *Neopestalotiopsis*; country: China; stateProvince: Hainan; locality: Haikou City, Xinglong Tropical Botanical Garden; verbatimCoordinates: 110°11’ E, 18°44’ N; recordedBy: Yu-ke He; associatedOccurrences: GUCC 21003; identifiedBy: Yu-ke He; dateIdentified: 2020; collectionID: HGUP 10003; occurrenceID: D0BFF7CB-F047-5DDC-9F03-121D9E38E95C

Description

Associated with leaf spots of *Salacca zalacca* (Gaertn.) Voss. Disease symptom: a small oval scab, ring-like, the inner ring is light brown to dark brown and the outer ring is light brown, the boundary is obvious, dark brown. A few black, small, isolated and punctuate conidia irregularly distributed on the scab. Sexual state: unknown. Asexual morph (Fig. 4): Conidiomata is dark, oblate, scattered on the host scab, 70−180 µm. Conidiophores discrete to lageniform or globular, hyaline, smooth– and thin–walled, simple and short. Conidia 18−23 × 6−7.5 µm, fusiform to clavate, straight to slightly, 4–septate; basal cell obconic with a truncate base, hyaline or pale brown, smooth– and thin–walled, 3.5−5 µm long; three median cells 12.5−15 µm long, light brown or hyaline, septa and periclinal walls darker than rest of the cell, wall rugose; second cell 4.5−5.5 µm long; third cell 4−5.5 µm long; fourth cell 5−6 µm long; apical cell 3–4.5 µm long, hyaline, conic to acute, with 1–2 tubular appendages inserted at different loci, but in the same crest at the apex of the apical cell, unbranched, flexuous, 12−20 µm long. The spores have tubular appendages or single appendage, unbranched, centric, 3.5−6 µm long.

Culture characteristics: Colonies on PDA medium reaching 4.5–5 cm diam. After 9 d at 24°C, odourless, without exudates, with black dots in the centre (conidiomata), the mycelium is white, soft and round with regular edges; reverse yellow to white. Under the surface of hyphal layer, releasing many conidia in a black, slimy mass.

Notes

Phylogenetically, isolated GUCC 21003 clustered with the ex-type strain of *N. samarangensis* (MFLUCC 12−0233). In morphology, our strain is very similar to *N. samarangensis* (Maharachchikumbura et al. 2013). A comparison of DNA bases (Table 2) demonstrated that the differences between these two strains are minute. Therefore, we concluded that they are the same species, but occurring on different hosts (*N. samarangensis* GUCC 21003 on leaf of *Salacca zalacca* vs. *N. samarangensis* MFLUCC 12-0233 on *Syzygium samarangense*).

Analysis

Phylogenetic analysis

The final concatenated alignment comprised 1809 characters including 65 taxa. The combined dataset contained 1352 constant, 253 parsimony uninformative and 204 parsimony informative characters. According to different optimisation criteria, the tree topology was similar, so the individual datasets were congruent and could be combined. There were two equally parsimonious trees from MP analysis and we chose the best one to show the topology (Fig. 1) (TL = 855, CI = 0.680, RI = 0.651, RC = 0.442, HI = 0.320). *Neopestalotiopsis elaeagni* (GUCC 21002) is a sister taxon of *N. chrysea* and *N. umbrinospora* with high support (MP-BS = 90%/96% ML-BS = 96% BYPP = 0.98). *Neopestalotiopsis zingiberis* (GUCC 21001) is a sister taxon of *N. magna* (MFLUCC 12–0652) only with high BI support (PP = 0.96). GUCC 21003 was closer to the ex-type strain of *N. samarangensis* (MFLUCC 12-0233T) with high BI support (PP = 0.98). The base-pair differences amongst the three new collections are listed in Table 2. It showed that *N.*

elaeagni (GUCC 21002), *N. chrysea* (MFLUCC 12-0261)*, N.umbrinospora* (MFLUCC 12-0285) and *N.asiatica* (MFLUCC 12-0286), differ by only one character difference in the ITS region, 19-27 characters in TEF1 and 2-5 characters in TUB2. Between *N. zingiberis* (GUCC 21001) and *N. magna* (MFLUCC 12-0652), there were 16 character differences in the ITS region, three characters in TEF1 and 35 characters in TUB2. Between *N. samarangensis* (GUCC 21003) and *N. samarangensis* (MFLUCC 12-0233), there was only one character difference in the ITS, nine characters in TEF1 and two characters in TUB2.

Discussion

In this study, we describe two new species and one new host record from China, namely *Neopestalotiopsis elaeagni*, *N. zingiberis* and *N. samarangensis*, based on morphological and phylogenetic analyses. For the morphology, we chose several indicators for the classification of *Neopestalotiopsis,* such as the size of conidia, the number and length of apical appendages and the basal appendage length (Maharachchikumbura et al. 2014a). For the phylogeny, we found that the different gene segments can distinguish the different inter-species relationships in *Neopestalotiopsis* (Table 2). However, some differences lacked significant variation to clearly distinguish the species of *Neopestalotiopsis*, such as the length and colour of the three median cells, the number of basal appendages and the ITS sequence data of *N. elaeagni* and *N. chrysea*. Therefore, we needed to combine the morphology and the phylogeny data to identify the new species.

China has reported 55 fungal diseases on 10 species of Zingiberaceae, including new diseases (Qi and Jiang 1994). However, most of the species have been identified, based on morphology alone. Most studies focused on the secondary products of fungi in Zingiberaceae and little research has been done on the diversity of fungi in Zingiberaceae (Taechowisan et al. 2003, Ginting et al. 2013, Anisha and Radhakrishnan 2017, Gupta et al. 2022). *Neopestalotiopsis* have been found on many different hosts and plant families (Maharachchikumbura et al. 2014b, Hyde et al. 2020), but few species have been found on Zingiberaceae in China. Therefore, in future work, comprehensive studies on Zingiberaceous *Neopestalotiopsis* will result in many more species being described in China.

We were unable to conduct the pathogenicity test in this research, although the *N. elaeagni* and *N. zingiberis* were isolated from the leaf spots. On the future work, similar to other relevant fields in mycology, it is necessary to identify the pathogenic taxa to the species level (Jayawardena et al. 2021), as it can help us to prevent diseases caused by them and reduce economic losses.

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References

- Akinsanmi OA, Nisa S, Jeff-Ego OS, Shivas RG, Drenth A (2017) Dry flower disease of *Macadamia* in Australia caused by *Neopestalotiopsis macadamiae* sp. nov. and *Pestalotiopsis macadamiae* sp. nov. Plant Disease 101 (1): 45-53. https://doi.org/10.1094/PDIS-05-16-0630-RE
- Anisha C, Radhakrishnan E (2017) Metabolite analysis of endophytic fungi from cultivars of *Zingiber officinale* Rosc. identifies myriad of bioactive compounds including tyrosol. 3 Biotech 7 (2): 1-10. https://doi.org/10.1007/s13205-017-0768-8
- Bezerra JDP, Machado AR, Firmino AL, Rosado AWC, Souza CAFd, Souza-Motta CMd, Freire KTLdS, Paiva LM, Magalhães OMC, Pereira OL (2018) Mycological diversity description I. Acta Botanica Brasilica 32: 656-666. https://doi.org/10.1590/0102-33062018abb0154
- Bruen TC, Philippe H, Bryant D (2006) A simple and robust statistical test for detecting the presence of recombination. Genetics 172 (4): 2665-2681. https://doi.org/10.1534/genetics.105.048975
- Carbone I, Kohn LM (1999) A method for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91 (3): 553-556. https://doi.org/10.2307/3761358
- Crous P, Wingfield M, Le Roux J, Richardson D, Strasberg D, Shivas R, Alvarado P, Edwards J, Moreno G, Sharma R (2015) Fungal Planet Description Sheets: 371-399. Persoonia 35: 264-327. https://doi.org/10.3767/003158514X682395
- Crous PW, Wingfield MJ, Roux JJL, Richardson DM, Strasberg D, Shivas RG, Alvarado P, Edwards J, Moreno G, Sharma R, Sonawane MS, Tan YP, Altés A, Barasubiye T, Barnes CW, Blanchette RA, Boertmann D, Bogo A, Carlavilla JR, Cheewangkoon R, Daniel R, de Beer ZW, Yáñez-Morales MdJ, Duong TA, Fernández-Vicente J, Geering AD, Guest DI, Held BW, Heykoop M, Hubka V, Ismail AM, Kajale SC, Khemmuk W, Kolařík M, Kurli R, Lebeuf R, Lévesque CA, Lombard L, Magista D, Manjón JL, Marincowitz S, Mohedano JM, Nováková A, Oberlies NH, Otto EC, Paguigan ND, Pascoe IG, Pérez-Butrón JL, Perrone G, Rahi P, Raja HA, Rintoul T, Sanhueza RM, Scarlett K, Shouche YS, Shuttleworth LA, Taylor PW, Thorn RG, Vawdrey LL, Solano-Vidal R, Voitk A, Wong PT, Wood AR, Zamora JC, Groenewald JZ (2015) Fungal Planet description sheets: 371–399. Persoonia - Molecular Phylogeny and Evolution of Fungi 35 (1): 264-327. https://doi.org/10.3767/003158515x690269

- Dissanayake A, Bhunjun C, Maharachchikumbura S, Liu J (2020) Applied aspects of methods to infer phylogenetic relationships amongst fungi. Mycosphere 11 (1): 2652-2676. https://doi.org/10.5943/mycosphere/11/1/18
- Freitas EF, Silva Md, Barros MV, Kasuya MC (2019) *Neopestalotiopsis hadrolaeliae* sp. nov., a new endophytic species from the roots of the endangered orchid *Hadrolaelia jongheana* in Brazil. Phytotaxa 416 (3): 211-220. https://doi.org/10.11646/phytotaxa.416.3.2
- Ginting RCB, Sukarno N, Widyastuti U, Darusman LK, KANAYA S (2013) Diversity of endophytic fungi from red ginger (*Zingiber officinale* Rosc.) plant and their inhibitory effect to *Fusarium oxysporum* plant pathogenic fungi. HAYATI Journal of Biosciences 20 (3): 127-137. https://doi.org/10.4308/hjb.20.3.127
- Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61 (4): 1323-1330. https://doi.org/10.1002/bit.260460112
- Gupta S, Choudhary M, Singh B, Singh R, Dhar MK, Kaul S (2022) Diversity and biological activity of fungal endophytes of *Zingiber officinale* Rosc. with emphasis on *Aspergillus terreus* as a biocontrol agent of its leaf spot. Biocatalysis and Agricultural Biotechnology 39 https://doi.org/10.1016/j.bcab.2021.102234
- Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. 41. Nucleic Acids Symposium Series. Information Retrieval Ltd., London, 3 pp. https://doi.org/10.1021/bk-1999-0734.ch008
- Huanaluek N, Jayawardena RS, Maharachchikumbura SSN, Harishchandra DL (2021) Additions to pestalotioid fungi in Thailand: *Neopestalotiopsis hydeana* sp. nov. and *Pestalotiopsis hydei* sp. nov. Phytotaxa 479 (1): 43-23. https://doi.org/10.11646/phytotaxa.479.1.2
- Huson DH (1998) SplitsTree: analyzing and visualizing evolutionary data. Bioinformatics 14 (1): 68-73. https://doi.org/10.1093/bioinformatics/14.1.68
- Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23 (2): 254-267. https://doi.org/10.1093/molbev/msj030
- Hyde KD, Jeewon R, Chen Y, Bhunjun CS, Calabon MS, Jiang H, Lin C, Norphanphoun C, Sysouphanthong P, Pem D (2020) The numbers of fungi: is the descriptive curve flattening? Fungal Diversity 103 (1): 219-271. https://doi.org/10.1007/s13225-020-00458-2
- Jayawardena R, Hyde K, McKenzie E, Jeewon R, Phillips A, Perera R, de Silva N, Maharachchikumbura S, Samarakoon M, Ekanayaka A, Tennakoon D, Dissanayake A, Norphanphoun C, Lin C, Manawasinghe I, Tian Q, Brahmanage R, Chomnunti P, Hongsanan S, Wang Y (2019) One stop shop III: taxonomic update with molecular phylogeny for important phytopathogenic genera: 51–75 (2019). Fungal Diversity 98: 77-160. https://doi.org/10.1007/s13225-019-00433-6
- Jayawardena R, Hyde K, de Farias ARG, Bhunjun C, Ferdinandez H, Manamgoda D, Udayanga D, Herath I, Thambugala K, Manawasinghe I, Gajanayake A, Samarakoon B, Bundhun D, Gomdola D, Huanraluek N, Sun Y, Tang X, Promputtha I, Thines M (2021) What is a species in fungal plant pathogens? Fungal Diversity 109 (1): 239-266. https://doi.org/10.1007/s13225-021-00484-8
- Jayawardena RS, Liu M, Maharachchikumbura SS, Zhang W, Xing Q, Hyde KD, Nilthong S, Li X, Yan J (2016) *Neopestalotiopsis vitis* sp. nov. causing grapevine leaf spot in China. Phytotaxa 258 (1): 74-63. https://doi.org/10.11646/phytotaxa.258.1.4

- Jayawardena RS, Hyde KD, McKenzie EH, Jeewon R, Phillips AJ, Perera RH, de Silva NI, Maharachchikumburua SS, Samarakoon MC, Ekanayake AH (2019) One stop shop III: taxonomic update with molecular phylogeny for important phytopathogenic genera: 51–75 (2019). Fungal Diversity 98 (1): 77-160. https://doi.org/10.1007/s13225-019-00433-6
- Jiang N, Bonthond G, Fan X, Tian C (2018) *Neopestalotiopsis rosicola* sp. nov. causing stem canker of *Rosa chinensis* in China. Mycotaxon 133 (2): 271-283. https://doi.org/10.5248/133.271
- Jiang N, Fan X, Tian C (2021) Identification and characterization of leaf-inhabiting fungi from *Castanea* plantations in China. Journal of Fungi 7 (1): 64. https://doi.org/10.3390/jof7010064
- Katoh K, Standley DM (2016) A simple method to control over-alignment in the MAFFT multiple sequence alignment program. Bioinformatics 32 (13): 1933-1942. https://doi.org/10.1093/bioinformatics/btw108
- Kumar V, Cheewangkoon R, Gentekaki E, Maharachchikumbura SS, Brahmanage RS, Hyde KD (2019) *Neopestalotiopsis alpapicalis* sp. nov. a new endophyte from tropical mangrove trees in Krabi Province (Thailand). Phytotaxa 393 (3): 251-262. https://doi.org/10.11646/phytotaxa.393.3.2
- Larsson A (2014) AliView: a fast and lightweight alignment viewer and editor for large datasets. Bioinformatics 30 (22): 3276-3278. https://doi.org/10.1093/bioinformatics/btu531
- Li GJ, Hyde KD, Zhao RL, Hongsanan S, Abdel-Aziz FA, Abdel-Wahab MA, Alvarado P, Alves-Silva G, Ammirati JF, Ariyawansa HA (2016) Fungal diversity notes 253–366: taxonomic and phylogenetic contributions to fungal taxa. Fungal Diversity 78 (1): 1-237. https://doi.org/10.1007/s13225-016-0366-9
- Maharachchikumbura SS, Guo L, Chukeatirote E, Bahkali AH, Hyde KD (2011) *Pestalotiopsis*— morphology, phylogeny, biochemistry and diversity. Fungal Diversity 50 (1): 167-187. https://doi.org/10.1007/s13225-011-0125-x
- Maharachchikumbura SS, Guo L, Cai L, Chukeatirote E, Wu WP, Sun X, Crous PW, Bhat DJ, McKenzie EH, Bahkali AH (2012) A multi-locus backbone tree for *Pestalotiopsis*, with a polyphasic characterization of 14 new species. Fungal Diversity 56 (1): 95-129. https://doi.org/10.1007/s13225-012-0198-1
- Maharachchikumbura SS, Chukeatirote E, Guo L, Crous PW, Mckenzie EH, Hyde KD (2013) *Pestalotiopsis* species associated with *Camellia sinensis* (tea). Mycotaxon 123 (1): 47-61. https://doi.org/10.5248/123.47
- Maharachchikumbura SS, Guo L, Chukeatirote E, Hyde KD (2014a) Improving the backbone tree for the genus *Pestalotiopsis*; addition of *P. steyaertii* and *P. magna* sp. nov. Mycological Progress 13 (3): 617-624. https://doi.org/10.1007/s11557-013-0944-0
- Maharachchikumbura SS, Hyde KD, Groenewald JZ, Xu J, Crous PW (2014b) *Pestalotiopsis* revisited. Studies in Mycology 79 (1): 121-186. https://doi.org/10.1016/j.simyco.2014.09.005
- Maharachchikumbura SS, Hyde KD, Jones EG, McKenzie E, Bhat JD, Dayarathne MC, Huang S, Norphanphoun C, Senanayake IC, Perera RH (2016) Families of sordariomycetes. Fungal Diversity 79 (1): 1-317. https://doi.org/10.1007/s13225-016-0369-6
- Ma X, Maharachchikumbura SS, Chen B, Hyde KD, Mckenzie EH, Chomnunti P, Kang J (2019) Endophytic pestalotiod taxa in Dendrobium orchids. Phytotaxa 419 (3): 286-268. https://doi.org/10.11646/phytotaxa.419.3.2

- Norphanphoun C, Jayawardena R, Chen Y, Wen T, Meepol W, Hyde K (2019) Morphological and phylogenetic characterization of novel pestalotioid species associated with mangroves in Thailand. Mycosphere 10: 531-578. https://doi.org/10.5943/mycosphere/10/1/9
- Nozawa S, Yamaguchi K, Van Hop D, Phay N, Ando K, Watanabe K (2017) Identification of two new species and a sexual morph from the genus *Pseudopestalotiopsis*. Mycoscience 58 (5): 328-337. https://doi.org/10.1016/j.myc.2017.02.008
- Nylander JA, Ronquist F, Huelsenbeck JP, Nieves-Aldrey J (2004) Bayesian phylogenetic analysis of combined data. Systematic Biology 53 (1): 47-67. https://doi.org/10.1080/10635150490264699
- O'Donnell K, Cigelnik E (1997) Two divergent intragenomic rDNA ITS2 types within a monophyletic lineage of the fungusfusariumare nonorthologous. Molecular Phylogenetics and Evolution 7 (1): 103-116. https://doi.org/10.1006/mpev.1996.0376
- O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proceedings of the National Academy of Sciences 95 (5): 2044-2049. https://doi.org/10.1073/pnas.95.5.2044
- Qi P, Jiang Z (1994) Descriptions of new species and combination of *Cercospora* and allied genera on medicinal plants. Journal of South China Agricultural University 15: 14-21.
- Quaedvlieg W, Binder M, Groenewald J, Summerell B, Carnegie A, Burgess T, Crous P (2014) Introducing the consolidated species concept to resolve species in the Teratosphaeriaceae. Persoonia-Molecular Phylogeny and Evolution of Fungi 33 (1): 1-40. https://doi.org/10.3767/003158514X681981
- Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61 (3): 539-542. https://doi.org/10.1093/sysbio/sys029
- Senanayake I (2020) Morphological approaches in studying fungi: collection, examination, isolation, sporulation and preservation. Mycosphere 11 (1): 2678-2754. https://doi.org/10.5943/mycosphere/11/1/20
- Song Y, Geng K, HYDE KD, ZHAO W, Wei J, Kang J, Wang Y (2013) Two new species of *Pestalotiopsis* from Southern China. Phytotaxa 126 (1): 32-22. https://doi.org/10.11646/PHYTOTAXA.126.1.2
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30 (9): 1312-1313. https://doi.org/10.1093/bioinformatics/btu033
- Swofford DL (2002) PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods). Mac Version 3. 1. 1.
- Taechowisan T, Peberdy JF, Lumyong S (2003) Isolation of endophytic actinomycetes from selected plants and their antifungal activity. World Journal of Microbiology and Biotechnology 19 (4): 381-385. https://doi.org/10.1023/A:1023901107182
- Tibpromma S, Hyde KD, McKenzie EH, Bhat DJ, Phillips AJ, Wanasinghe DN, Samarakoon MC, Jayawardena RS, Dissanayake AJ, Tennakoon DS (2018) Fungal diversity notes 840–928: micro-fungi associated with Pandanaceae. Fungal Diversity 93 (1): 1-160. https://doi.org/10.1007/s13225-018-0408-6

- Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladistics 27 (2): 171-180. https://doi.org/10.1111/j.1096-0031.2010.00329.x
- White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications 18 (1): 315-322.
- Wijayawardene N, Hyde K, Al-Ani LKT, Tedersoo L, Haelewaters D, Becerra AG, Schnittler M, Shchepin O, Novozhilov Y, Silva-Filho A (2020) Outline of fungi and fungus-like taxa. Mycosphere 11 (1): 1160-1456. https://doi.org/10.5943/mycosphere/11/1/8
- Zhang Z, Liu R, Liu S, Mu T, Zhang X, Xia J (2022) Morphological and phylogenetic analyses reveal two new species of *Sporocadaceae* from Hainan, China. MycoKeys 88 https://doi.org/10.3897/mycokeys.88.82229

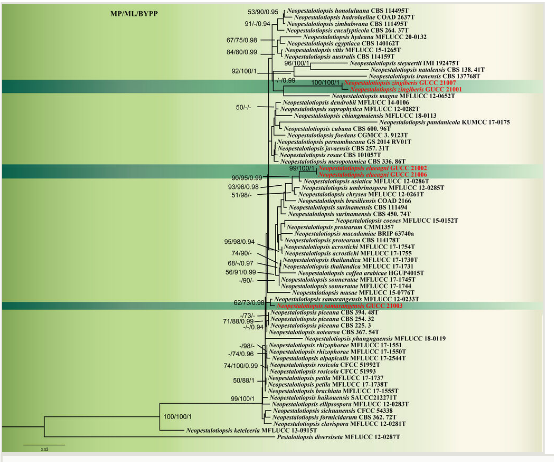

Figure 1.

Consensus phylogram of 1,000 trees resulting from an RAxML analysis of the (ITS+TUB2+TEF1) alignment of the analysed *Neopestalotiopsis* sequences. *Pestalotiopsis diversiseta* (MFLUCC 12–0287) is used as the outgroup taxon. The MP bootstrap values ≥ 50%, ML bootstraps ≥ 70% and Bayesian posterior probabilities ≥ 0.90 (MPBS/MLBS/PPBY) are given at the nodes. New collections obtained in this study are in red.

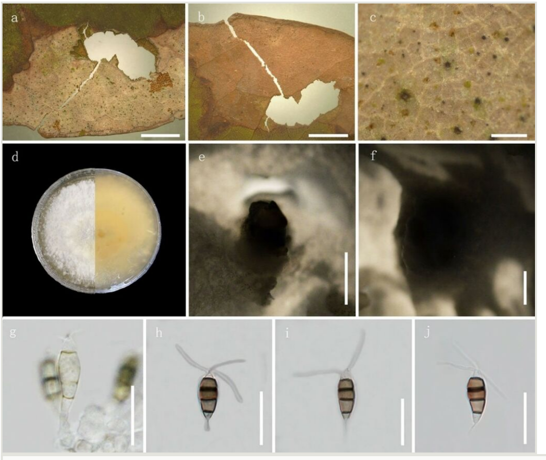

Figure 2.

Neopestalotiopsis elaeagni (Specimen code: HGUP 10002). **a–c** Appearance on host surface; **d** Colony top view and reverse view; **e–f** Conidiomata on PDA; **g** Conidiogenous cells; **h–j** Conidia. Scale bars: **a–b** = 10 mm, **c** = 1 mm, **e–f** = 500 μm, **g–j** = 20 μm.

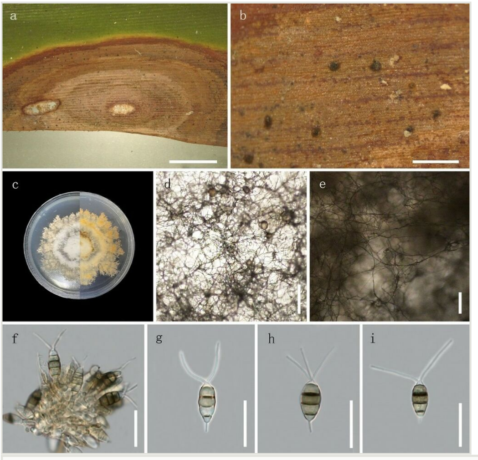

Figure 3.

Neopestalotiopsis zingiberis (Specimen code: HGUP 10001). **a–b** Appearance on host surface; **c** Colony top view and reverse view; **d–e** Mycelium; **f** Conidiogenous cells; **g–i** Conidia. Scale bars: **a** =10 mm, **b** = 1 mm, **d–e** = 200 μm, **f–i** = 20 μm.

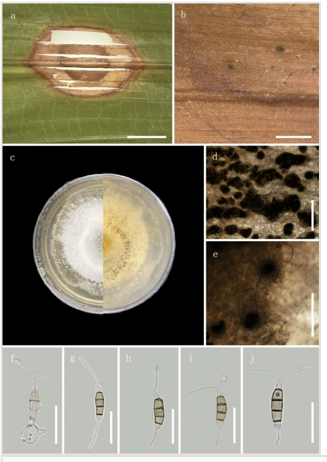

Figure 4.

Neopestalotiopsis samarangensis (Specimen code: HGUP 10003). **a–b** Appearance on host surface; **c** Colony top view and reverse view; **d–e** Conidiomata on PDA; **f** Conidiogenous cells; **g–j** Conidia. Scale bars: **a** = 10 mm, **b** = 1 mm, **d–e** = 500 μm, **f–j** = 20 μm.

Table 1.

GenBank accession numbers of molecular phylogenetic analyses. Ex–type isolates are labelled with superscript T. The new isolates are in bold.

Taxa	Strain number	Host	Country	ITS	TUB2	TEF1	Reference
Neopestalotiopsis acrostichi	MFLUCC 17–1754^{T}	*Acrostichum aureum*	Thailand	MK764272	MK764338	MK764316	Norphanphoun et al. 2019
N. acrostichi	MFLUCC 17–1755	*Acrostichum aureum*	Thailand	MK764273	MK764339	MK764317	Norphanphoun et al. 2019
N. alpapicalis	MFLUCC 17–2544^{T}	*Rhyzophora mucronata*	Thailand	MK357772	MK463545	MK463547	Kumar et al. 2019
N. aotearoa	CBS 367.54^{T}	*Canvas*	New Zealand	KM199369	KM199454	KM199526	Maharachchikumbura et al. 2014b
N. asiatica	MFLUCC 12–0286^{T}	unidentified tree	China	JX398983	JX399018	JX399049	Maharachchikumbura et al. 2012
N. australis	CBS 114159T	*Telopea* sp.	Australia	KM199348	KM199432	KM199537	Maharachchikumbura et al. 2014b
N. brachiata	MFLUCC 17–1555^{T}	*Rhizophora apiculata*	Thailand	MK764274	MK764340	MK764318	Norphanphoun et al. 2019
N. brasiliensis	COAD 2166	*Psidium guajava*	Brazil	MG686469	MG692400	MG692402	Bezerra et al. 2018
N. chiangmaiensis	MFLUCC 18–0113	*Pandanaceae*	Thailand	–	MH412725	MH388404	Tibpromma et al. 2018
N. chrysea	MFLUCC 12–0261^{T}	dead leaves	China	JX398985	JX399020	JX399051	Maharachchikumbura et al. 2012
N. clavispora	MFLUCC 12–0281^{T}	*Magnolia* sp.	China	JX398979	JX399014	JX399045	Maharachchikumbura et al. 2012
N. cocoes	MFLUCC 15–0152^{T}	*Cocos nucifera*	Thailand	NR156312	–	KX789689	Norphanphoun et al. 2019
N. coffea–arabicae	HGUP4015^{T}	*Coffea arabica*	China	KF412647	KF412641	KF412644	Song et al. 2013
N. cubana	CBS 600.96^{T}	leaf litter	Cuba	KM199347	KM199438	KM199521	Maharachchikumbura et al. 2014b
N. dendrobii	MFLUCC 14–0106	*Dendrobium cariniferum*	Thailand	MK993571	MK975835	MK975829	Ma et al. 2019
N. egyptiaca	CBS 140162^{T}	*Mangifera indica*	Egypt	KP943747	KP943746	KP943748	Crous et al. 2015
N. elaeagni	HGUP10002 HGUP10004	*Elaeagnus pungens*, *Elaeagnus pungens*	China China	**MW930716** **ON597079**	**MZ683391** **ON595537**	**MZ203452** **ON595535**	**This study** **This study**
N. ellipsospora	MFLUCC 12–0283^{T}	dead plant material	China	JX398980	JX399016	JX399047	Maharachchikumbura et al. 2012
N. eucalypticola	CBS 264.37^{T}	*Eucalyptus globulus*	–	KM199376	KM199431	KM199551	Maharachchikumbura et al. 2014b

N. foedans	CGMCC 3.9123 ^{T}	unidentified mangrove plant	China	JX398987	JX399022	JX399053	Maharachchikumbura et al. 2012
N. formicidarum	CBS 362.72^{T}	dead ant	Ghana	KM199358	KM199455	KM199517	Maharachchikumbura et al. 2014b
N. hadrolaeliae	COAD 2637^{T}	*Hadrolaelia jongheana*	Brazil	MK454709	MK465120	MK465122	Freitas et al. 2019
N. haikouensis	SAUCC212271^{T}	*Ilexchinensis*	China	OK087294	OK104870	OK104877	Zhang et al. 2022
N. honoluluana *N. hydeana*	CBS 114495^{T} MFLUCC 20–0132	*Telopea* sp. *Artocarpus heterophyllus*	USA Thailand	KM199364 MW266069	KM199457 MW251119	KM199548 MW251129	Maharachchikumbura et al. 2014b Huanaluek et al. 2021
N. iranensis	CBS 137768^{T}	*Fragaria* × *ananassa*	Iran	KM074048	KM074057	KM074051	Song et al. 2013
N. javaensis	CBS 257.31^{T}	*Cocos nucifera*	Indonesia	KM199357	KM199437	KM199543	Maharachchikumbura et al. 2014b
N. keteleeria	MFLUCC 13–0915^{T}	*Keteleeria pubescens*	China	KJ503820	KJ503821	KJ503822	Song et al. 2013
N. magna	MFLUCC 12–0652^{T}	*Pteridium* sp.	France	KF582795	KF582793	KF582791	Maharachchikumbura et al. 2012
N. macadamiae	BRIP 63740a	*Macadamia* sp.	Australia	KX186617	KX186656	KX186628	Akinsanmi et al. 2017
N. mesopotamica	CBS 336.86^{T}	*Pinus brutia*	Iraq	KM199362	KM199441	KM199555	Maharachchikumbura et al. 2014b
N. musae	MFLUCC 15–0776^{T}	*Musa* sp.	Thailand	NR156311	KX789686	KX789685	Li et al. 2016
N. natalensis	CBS 138.41^{T}	*Acacia mollissima*	South Africa	NR156288	KM199466	KM199552	Maharachchikumbura et al. 2014b
N. pandanicola	KUMCC 17–0175	*Pandanaceae*	China	–	MH412720	MH388389	Tibpromma et al. 2018
N. pernambucana	GS 2014 RV01^{T}	*Vismia guianensis*	Brazil	KJ792466	–	–	Maharachchikumbura et al. 2014b
N. petila	MFLUCC 17–1738^{T}	*Rhizophora mucronata*	Thailand	MK764275	MK764341	MK764319	Norphanphoun et al. 2019
N. petila	MFLUCC 17–1737	*Rhizophora mucronata*	Thailand	MK764276	MK764342	MK764320	Norphanphoun et al. 2019
N. phangngaensis	MFLUCC 18–0119	*Pandanaceae*	Thailand	MH388354	MH412721	MH388390	Tibpromma et al. 2018
N. piceana	CBS 394.48^{T}	*Picea* sp.	UK	KM199368	KM199453	KM199527	Maharachchikumbura et al. 2014b
N. piceana	CBS 254.32	*Cocos nucifera*	Indonesia	KM199372	KM199452	KM199529	Maharachchikumbura et al. 2014b
N. piceana	CBS 225.3	*Mangifera indica*	–	KM199371	KM199451	KM199535	Maharachchikumbura et al. 2014b
N. protearum	CBS 114178^{T}	*Leucospermum cuneiforme* cv. *Sunbird*	Zimbabwe	JN712498	KM199463	KM199542	Maharachchikumbura et al. 2014b

N. protearum	CMM1357	–	–	KY549597	KY549632	KY549594	Maharachchikumbura et al. 2014b
N. rhizophorae	MFLUCC 17–1550[T]	*Rhizophora mucronata*	Thailand	MK764277	MK764343	MK764321	Norphanphoun et al. 2019
N. rhizophorae	MFLUCC 17–1551	*Rhizophora mucronata*	Thailand	MK764278	MK764344	MK764322	Norphanphoun et al. 2019
N. rosae	CBS 101057[T]	*Rosa* sp.	New Zealand	KM199359	KM199429	KM199523	Maharachchikumbura et al. 2014b
N. rosicola	CFCC 51992[T]	*Rosa chinensis*	China	KY885239	KY885245	KY885243	Jiang et al. 2018
N. rosicola	CFCC 51993	*Rosa chinensis*	China	KY885240	KY885246	KY885244	Jiang et al. 2018
N. samarangensis	MFLUCC 12–0233[T]	*Syzygium samarangense*	Thailand	JQ968609	JQ968610	JQ968611	Maharachchikumbura et al. 2012
N. samarangensis	HGUP10003	*Salacca zalacca*	China	**MW930717**	**MZ683392**	**MZ540914**	**This study**
N. saprophytica	MFLUCC 12–0282[T]	*Magnolia* sp.	China	KM199345	KM199433	KM199538	Maharachchikumbura et al. 2012
N. sichuanensis	CFCC 54338 = SM15-1[T]	*Castanea mollissima*	China	MW166231	MW218524	MW199750	Jiang et al. 2021
N. sonneratae	MFLUCC 17–1745[T]	*Sonneronata alba*	Thailand	MK764279	MK764345	MK764323	Norphanphoun et al. 2019
N. sonneratae	MFLUCC 17–1744	*Sonneronata alba*	Thailand	MK764280	MK764346	MK764324	Norphanphoun et al. 2019
N. steyaertii	IMI 192475[T]	*Eucalyptus viminalis*	Australia	KF582796	KF582794	KF582792	Maharachchikumbura et al. 2012
N. surinamensis	CBS 450.74[T]	soil under *Elaeis guineensis*	Suriname	KM199351	KM199465	KM199518	Maharachchikumbura et al. 2014b
N. surinamensis	CBS 111494	*Protea eximia*	Zimbabwe	–	KM199462	KM199530	Maharachchikumbura et al. 2014b
N. thailandica	MFLUCC 17–1730[T]	*Rhizophora mucronata*	Thailand	MK764281	MK764347	MK764325	Norphanphoun et al. 2019
N. thailandica	MFLUCC 17–1731	*Rhizophora mucronata*	Thailand	MK764282	MK764348	MK764326	Norphanphoun et al. 2019
N. umbrinospora	MFLUCC 12–0285[T]	unidentified plant	China	JX398984	JX399019	JX399050	Maharachchikumbura et al. 2012
N. vitis	MFLUCC 15–1265[T]	*Vitis vinifera*	China	KU140694	KU140685	KU140676	Jayawardena et al. 2016
N. zimbabwana	CBS 111495[T]	*Leucospermum cunciforme*	Zimbabwe	JX556231	KM199456	KM199545	Norphanphoun et al. 2019
N. zingiberis	HGUP10001 HGUP10005	*Zingiber officinale*, *Zingiber officinale*	China China	**MW930715** **ON597078**	**MZ683390** **ON595538**	**MZ683389** **ON595536**	**This study** **This study**
Pestalotiopsis diversiseta	MFLUCC 12–0287[T]	dead plant material	China	NR120187	JX399040	JX399073	Maharachchikumbura et al. 2012

Table 2.

The differences of DNA bases on different gene regions between our strains. Our strains are in bold.

Species	Strain number	ITS (1–568)	TEF1 (569–1520)	TUB2 (1521–1972)
Neopestalotiopsis elaeagni *	GUCC 21002	0	0	0
Neopestalotiopsis chrysea	MFLUCC 12–0261	1	27	2
Neopestalotiopsis umbrinospora	MFLUCC 12–0285	1	25	4
Neopestalotiopsis asiatica	MFLUCC 12–0286	1	19	5
Neopestalotiopsis zingiberis *	GUCC 21001	0	0	0
Neopestalotiopsis magna	MFLUCC 12–0652	16	3	35
Neopestalotiopsis samarangensis *	GUCC 21003	0	0	0
Neopestalotiopsis samarangensis	MFLUCC 12–0233	1	9	2

Table 3.

Comparison of conidia of *Neopestalotiopsis* species related to this study. Our strains are in bold.

Species	**Strain**	**Conidial size (μm)**	**Apical appendages**		**Basal appendage Length (μm)**
			Number	Length (μm)	
N.chrysea	MFLUCC 12–0261	20–24 × 5.5–7	3	22–30	3–6
N. umbrinospora	MFLUCC 12–0285	19–29 × 6–8	1–3	22–35	5–7
N. asiatica	MFLUCC 12–0286	20–26 × 5–7	2–4	20–30	4–8
N. elaeagni *	GUCC 21002	19–25 × 4.5–7	1–3	13–30	5–7.5
	GUCC 21006				
N. magna	MFLUCC 12–0652	42–46 × 9.5–12	2–4	16–26	11–15
N. zingiber *	GUCC 21001	21–31 × 6–9.5	1–3	12–15	0–6
	GUCC 21007				